

# Isolation and Characterization of Two New Alkaloids, Norpandamarilactonine-A and -B, from *Pandanus amaryllifolius* by Spectroscopic and Synthetic Methods

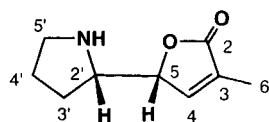
Hiromitsu Takayama,<sup>\*,†</sup> Tomotake Ichikawa,<sup>†</sup> Mariko Kitajima,<sup>†</sup> Maribel G. Nonato,<sup>‡</sup> and Norio Aimi<sup>†</sup>

Graduate School of Pharmaceutical Sciences, Chiba University, 1-33, Yayoi-cho, Inage-ku, Chiba 263-8522, Japan, and Research Center for the Natural Sciences, University of Santo Tomas, Espana, Manila 1008, Philippines

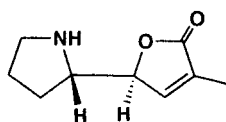
Received April 24, 2001

Two new alkaloids, norpandamarilactonine-A (**1**) and -B (**2**), which have a pyrrolidinyl- $\alpha,\beta$ -unsaturated  $\gamma$ -lactone moiety as in the known pandamarilactonine alkaloids, were isolated from the leaves of *Pandanus amaryllifolius*. Their structures were determined by spectroscopic analysis and total synthesis.

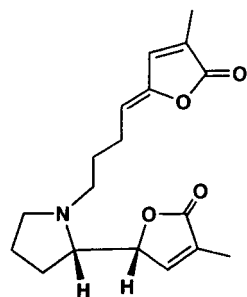
The genus *Pandanus* (Pandanaceae) comprises about 600 species which are widely distributed in tropical and subtropical regions. In a recent pharmacological survey, the hypoglycemic effect of an extract of *P. odoratus* was noted.<sup>1</sup> During our chemical studies on the secondary metabolites in *Pandanus* plants,<sup>2</sup> we reported the isolation of pandamarilactonines-A (**3**) and -B (**4**), pyrrolidine alkaloids from *P. amaryllifolius*.<sup>3</sup> Further investigation of the minor bases in fresh leaves of this plant resulted in the isolation of two additional alkaloids (**1** and **2**), whose structure elucidation by spectroscopic and synthetic methods are described herein.



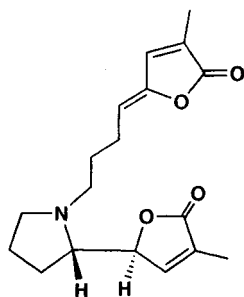
Norpandamarilactonine-A (**1**)



Norpandamarilactonine-B (**2**)



Pandamarilactonine-A (**3**)



Pandamarilactonine-B (**4**)

Compound **1** was obtained as an amorphous powder,  $[\alpha]_D^{19} 0^\circ$  (*c* 0.30,  $\text{CHCl}_3$ ), and high-resolution FABMS analysis established the molecular formula as  $\text{C}_9\text{H}_{13}\text{NO}_2$ . The presence of an  $\alpha$ -methyl- $\alpha,\beta$ -unsaturated  $\gamma$ -lactone residue was shown by characteristic signals in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra [ $\delta$  7.13 (1H, ddd,  $J = 0.8, 1.6, 1.6$  Hz, H-4), 4.73 (1H, ddd,  $J = 1.6, 1.9, 6.6$  Hz, H-5), 1.93 (3H);  $\delta$  174.3 (C-2), 130.7 (C-3), 147.7 (C-4), 83.8 (C-5), 10.7 (C-6)]. Using the residual four carbons (three methylenes and one methine) and one nitrogen atom, a pyrrolidine ring could be constructed. In the HMBC spectrum, the methine

proton ( $\delta$  3.18, 1H, ddd,  $J = 6.6, 6.6, 7.4$  Hz, H-2') on the pyrrolidine ring correlated with the  $\text{sp}^2$  carbon at C-4 ( $\delta$  147.7) in the  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone ring. In addition, the methine proton ( $\delta$  4.73) at C-5 ( $\delta$  83.8) in the  $\gamma$ -lactone ring had connectivity between C-2' and C-3' in the pyrrolidine ring. All the above findings enabled us to describe the molecular structure of the new alkaloid as **1**, a pyrrolidinyl- $\alpha,\beta$ -unsaturated  $\gamma$ -lactone skeleton, except for the stereochemistry of the vicinal asymmetric centers at C-5 and C-2'. Because of the lack of a  $\gamma$ -alkylidene- $\alpha,\beta$ -unsaturated  $\gamma$ -lactone moiety as in the known alkaloids, pandamarilactonines (**3** and **4**), we now name the new alkaloid (**1**) norpandamarilactonine-A.

Alkaloid **2** was also obtained as an amorphous powder, exhibiting  $[\alpha]_D^{19} 0^\circ$  (*c* 0.70,  $\text{CHCl}_3$ ). The UV and mass spectra, as well as the molecular formula obtained by HR-FABMS, were almost identical to those of **1**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were also very similar, indicating that **1** and **2** were diastereomeric at the C-5 and C-2' positions.

To confirm the structures and relative stereochemistry at C-5 and C-2' in the new alkaloids, we planned the total synthesis (Scheme 1). According to the procedure of Martin et al.,<sup>4</sup> compound **5** was prepared from 2-pyrrolidone and 3-methylfuran-2(5*H*)-one. The *threo* stereochemistry of the major product (**5**) obtained by vinylogous Mannich coupling reaction has been established by X-ray analysis.<sup>4</sup> The protecting group on the nitrogen in **5** was removed with TMSI in  $\text{CH}_3\text{CN}$  to give the secondary amine in 94% yield, which was identical with the natural product, norpandamarilactonine-B (**2**), by direct comparison of the chromatographic behavior and high-resolution MS and  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. Therefore, the relative stereochemistry of norpandamarilactonine-A (**1**) was determined to be *erythro*.

In summary, two new diastereomeric alkaloids (**1** and **2**) having a pyrrolidinyl- $\alpha,\beta$ -unsaturated  $\gamma$ -lactone skeleton were isolated as minor constituents from a tropical medicinal plant, *Pandanus amaryllifolius*. These interesting molecules possessing the substructure of the known alkaloids **3** and **4** were first characterized by spectroscopic analysis and then the structures were confirmed by total synthesis.

## Experimental Section

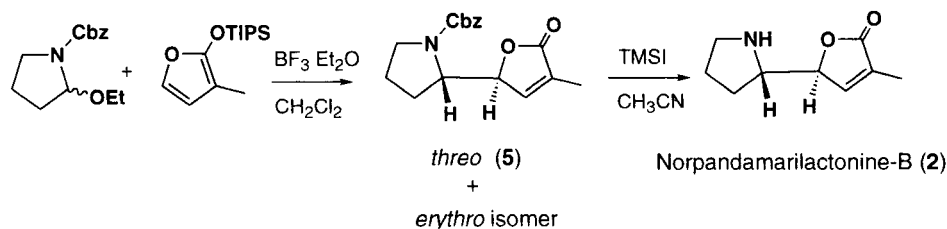
**General Experimental Procedures.** Optical rotations were measured on a JASCO DIP-140 polarimeter. UV and IR spectra were recorded on Hitachi U-3400 and JASCO FT/IR-230 spectrophotometers, respectively. EIMS and FABMS were

\* To whom correspondence should be addressed. Tel and Fax: (81) 43 2902902. E-mail: htakayam@p.chiba-u.ac.jp.

<sup>†</sup> Chiba University.

<sup>‡</sup> University of Santo Tomas.

## Scheme 1



recorded on JEOL JMS-AM20 and JEOL JMS-HX110 mass spectrophotometers, respectively. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR, COSY, HMQC, HMBC, and NOE were recorded on JEOL JNM A-500 and JEOL JNM ECP600 spectrometers. The chemical shifts are given in  $\delta$  (ppm) and coupling constants in Hz. Kieselgel 60 (Merck, 70–230 and 230–400 meshes) and a silica gel prepacked column (Kusano CPS-HS-221-05) were used for column chromatography.

**Plant Material.** The fresh leaves of *P. amaryllifolius* were purchased at a flower market in Bangkok (Thailand) and identified by Dr. Kittisak Likhitwitayawuid, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. A voucher specimen was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Chiba University.

**Extraction and Isolation of Alkaloids.** Fresh young leaves (8.0 kg) of *P. amaryllifolius* were macerated with EtOH (20 L) three times and filtered. The combined filtrates were concentrated under reduced pressure to give a crude extract (201 g), which was then partitioned between Et<sub>2</sub>O and 5% aqueous H<sub>2</sub>SO<sub>4</sub>. The water-soluble fraction was alkalized with concentrated NH<sub>4</sub>OH (pH 10) and exhaustively extracted with CHCl<sub>3</sub>. The organic layer was dried over MgSO<sub>4</sub> and evaporated to give a crude alkaloidal fraction (10.03 g). A portion of the crude base (1.63 g) was roughly separated by silica gel flash column chromatography using a CHCl<sub>3</sub>–MeOH/CHCl<sub>3</sub> gradient to give seven fractions. The 10% MeOH/CHCl<sub>3</sub> eluate was rechromatographed over SiO<sub>2</sub> using the same solvent to give 6 mg of norpandamarilactonine-A (1) and 33 mg of norpandamarilactonine-B (2).

**Norpandamarilactonine-A (1):** amorphous powder;  $[\alpha]_D^{19}$  0° (c 0.30); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 274 (0.44), 252 (0.35), 207 (2.29) nm; IR (neat)  $\nu_{\text{max}}$  1750 (lactone)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  7.13 (1H, ddd,  $J$  = 0.8, 1.6 and 1.6 Hz, H-4), 4.73 (1H, ddd,  $J$  = 1.6, 1.9 and 6.6 Hz, H-5), 3.18 (1H, ddd,  $J$  = 6.6, 6.6 and 7.4 Hz, H-2'), 2.96 (1H, ddd,  $J$  = 6.3, 6.3 and 10.4 Hz, H-5'), 2.93 (1H, ddd,  $J$  = 6.8, 6.8 and 10.4 Hz, H-5'), 1.93 (3H, s, H<sub>3</sub>-6), 1.84–1.92 (1H, m, H-3'), 1.72–1.90 (2H, m, H<sub>2</sub>-4'), 1.63 (1H, dddd,  $J$  = 6.3, 6.3, 6.6 and 12.9 Hz, H-3');  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$  174.3 (C-2), 147.7 (C-4), 130.7 (C-3), 83.8 (C-5), 60.4 (C-2'), 47.1 (C-5'), 27.9 (C-3'), 25.6 (C-4'), 10.7 (C-6); FABMS (NBA)  $m/z$  168 [M + H]<sup>+</sup>; HRFABMS (NBA)  $m/z$  168.1039 (calcd for C<sub>9</sub>H<sub>14</sub>NO<sub>2</sub>, 168.1025).

**Norpandamarilactonine-B (2):** amorphous powder;  $[\alpha]_D^{19}$  0° (c 0.70); UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 274 (0.36), 253 (0.29), 207 (2.58) nm; IR (neat)  $\nu_{\text{max}}$  1750 (lactone)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  7.02 (1H, ddd,  $J$  = 1.4, 1.7 and 3.0 Hz, H-4), 4.79

(1H, dddd,  $J$  = 1.6, 1.9, 3.0, and 6.6 Hz, H-5), 3.20 (1H, ddd,  $J$  = 6.6, 7.1, and 7.4 Hz, H-2'), 2.98 (1H, ddd,  $J$  = 5.8, 7.1 and 12.9 Hz, H-5'), 2.91 (1H, ddd,  $J$  = 6.6, 7.7, and 14.3 Hz, H-5'), 1.93 (3H, s, H<sub>3</sub>-6), 1.87 (1H, dddd,  $J$  = 3.0, 7.4, 10.7, and 15.4 Hz, H-3'), 1.81 (1H, m, H-4'), 1.74 (1H, m, H-4'), 1.56 (1H, dddd,  $J$  = 5.2, 6.9, 7.1 and 15.4 Hz, H-3');  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$  174.1 (C-2), 146.6 (C-4), 131.2 (C-3), 84.3 (C-5), 60.2 (C-2'), 46.5 (C-5'), 26.8 (C-3'), 25.1 (C-4'), 10.7 (C-6); FABMS (NBA)  $m/z$  168 [M + H]<sup>+</sup>; HRFABMS (NBA)  $m/z$  168.1030 (calcd for C<sub>9</sub>H<sub>14</sub>NO<sub>2</sub>, 168.1025).

**Synthesis of Norpandamarilactonine-B (2).** To a solution of diastereomerically pure carbamate (5) (30.6 mg, 0.1 mmol), which was prepared according to the procedure by Martin,<sup>4</sup> in CH<sub>3</sub>CN (1 mL), was added TMSI (43  $\mu\text{L}$ , 0.3 mmol) at –10 °C under argon. The reaction mixture was stirred at the same temperature for 15 min and then stirred at 0 °C for 15 min. The reaction mixture was poured into a chilled solution of 1 N HCl, and the whole mixture was extracted with Et<sub>2</sub>O. The aqueous layer was basified with 1 N NaOH, and the mixture was extracted with CHCl<sub>3</sub> three times. The combined organic layers were washed with H<sub>2</sub>O, dried over MgSO<sub>4</sub>, and evaporated to give 2 (16 mg, 94%), which was identical to natural norpandamarilactonine-B by comparison of their chromatographic behaviors, UV,  $^1\text{H}$  and  $^{13}\text{C}$  NMR, and mass spectra.

**Acknowledgment.** This work was supported in part by a Grant-in-Aid (No. 10877349) for Scientific Research from the Ministry of Education, Science, Sports, and Culture, Japan.

## References and Notes

- (1) (a) Peungvicha P.; Thirawarapan S. S.; Watanabe H. *Biol. Pharm. Bull.* **1996**, *19*, 364–366. (b) Peungvicha P.; Thirawarapan S. S.; Watanabe H. *Jpn. J. Pharmacol.* **1998**, *78*, 395–398. (c) Peungvicha P.; Temsiriririkkul, R.; Prasain, J. K.; Tezuka, Y.; Kadota, S.; Thirawarapan S. S.; Watanabe H. *J. Ethnopharmacol.* **1998**, *62*, 79–84.
- (2) (a) Nonato, M. G.; Garson, M. J.; Truscott, R. J. W.; Carver, J. A. *Phytochemistry* **1993**, *34*, 1159–1163. (b) Takayama, H.; Kuwajima, T.; Kitajima, M.; Nonato, M. G.; Aimi, N. *Heterocycles* **1999**, *50*, 75–78. (c) Takayama, H.; Kuwajima, T.; Kitajima, M.; Nonato, M. G.; Aimi, N. *Nat. Med.* **1999**, *53*, 335. (d) Takayama, H.; Ichikawa, T.; Kitajima, M.; Aimi, N.; Lopez, D.; Nonato, M. G. *Tetrahedron Lett.* **2001**, *42*, 2995–2996.
- (3) Takayama, H.; Ichikawa, T.; Kuwajima, T.; Kitajima, M.; Seki, H.; Aimi, N.; Nonato, M. G. *J. Am. Chem. Soc.* **2000**, *122*, 8635–8639.
- (4) Martin, S. F.; Barr, K. J.; Smith, D. W.; Bur, S. K. *J. Am. Chem. Soc.* **1999**, *121*, 6990–6997.

NP010213H